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Fast NMR Screening of Macromolecular Complexes by a Paramagnetic Spin Relaxation Filter

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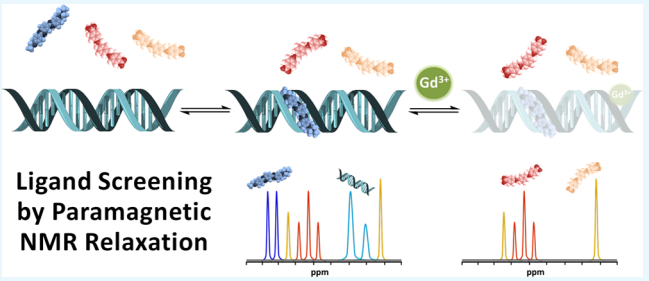
Supporting Information

ABSTRACT: The paramagnetic spin relaxation filter is described for the rapid NMR screening of intermolecular interactions between ligands and macromolecular anionic receptors with large transverse relaxation enhancements (R_{2p}). The addition of micromolar concentrations of Gd^{3+} to the mixture produces the immediate broadening/suppression of the NMR signals of interacting species while leaving unaffected those of noncompetitive binders (one-dimensional and two-dimensional experiments). The method is highly sensitive, unveiling interactions that are too weak to generate changes in chemical shifts or relaxation times. It is operationally very simple and hence, it is amenable to ready implementation by nonspecialists. Examples of application such as detecting the formation of interpolymer complexes, cyclodextrin host−guest interactions, and the screening of DNA ligands are included that demonstrate the reliability and broad applicability of the method.



INTRODUCTION

NMR is a powerful tool for the study of intermolecular interactions in drug discovery and supramolecular chemistry. Various NMR-sensitive parameters that change upon binding (chemical shifts, relaxation and diffusion rates, nuclear Overhauser effect (NOE), or saturation transfer effects) can be exploited as measures of the process.[1] The enhancement of the transverse relaxation rate ($R_2 = 1/T_2$, where T_2 is the transverse relaxation time) of low molecular weight ligands binding to macromolecular receptors has been widely exploited in recognition studies.[2] The proportionality between R_2 and the spectral linewidth[3] produces a selective broadening in the resonances of binding ligands, which might even be perceived in one-dimensional (1D) ^{1}H experiments. For small receptors and low affinity interactions, where broadening goes unnoticed, sensitivity can be improved via filtered experiments (T_2, or $T_1\rho$, the spin-lattice relaxation time in the rotating frame).[4] Still, the identification of binding is not always straightforward due to small relaxation enhancements. To overcome this shortcoming, Jahnke[5] and others[6] have described spin labels covalently bound to protein receptors. This approach takes advantage of the faster relaxation of nuclei in paramagnetic environments,[7] an effect that is proportional to the distance between the spin label and the active site where the ligand interacts. Related strategies exploiting the paramagnetism of lanthanides complexed to proteins[8] or ligands[9] have also been described to determine the three-dimensional structure of protein−ligand complexes by analysis of pseudocontact shifts. Such schemes are, however, very laborious for routine ligand screening. Not only does the paramagnetic probe need to be covalently bound in advance, but this must also occur in the proximity of the binding site/epitope. In this context, the development of faster and more user-friendly NMR screening technologies is highly demanded, especially for direct application by nonspecialists.

Our group has recently described the use of Gd^{3+} ($S = 7/2$, the largest spin moment among the elements; a high electronic correlation time, τ_s, of ca. 10^{-8} s) as a paramagnetic spin relaxation (PSR) agent for the selective signal broadening/suppression of certain components in mixtures according to their Gd^{3+}-complexing ability (^{1}H and ^{13}C PSR filter).[10,11] The method relies on the faster R_2 of species in chemical exchange with Gd^{3+},[12] and it is compatible with traditional relaxation and diffusion filters. The R_2 of nuclear spins in a paramagnetic environment is given by $R_2 = R_{2d} + cR_{2p}$, where R_{2d} is the transverse relaxation rate in the absence of paramagnetic effects ($R_{2d} = 1/T_{2d}$), c is the concentration of the paramagnetic agent, and R_{2p} is the transverse relaxation enhancement in the presence of the paramagnetic agent.[13] We have disclosed that the PSR filter is dominated by R_{2p} (values in the range 0.1−20 000 s^{-1} mM^{-1}, D_2O, 500 MHz) rather than the original R_{2d} (T_{2d}) values, so that R_{2p} represents a reliable and predictive tool for selective PSR suppressions.[11] The higher the R_{2p} of a component in a mixture, the easier its selective suppression in 1D and two-dimensional (2D) PSR experiments. Bearing in mind that anionic macromolecules (species with $R_{2p} > 1000$ s^{-1} mM^{-1}) can be suppressed in the presence of any small

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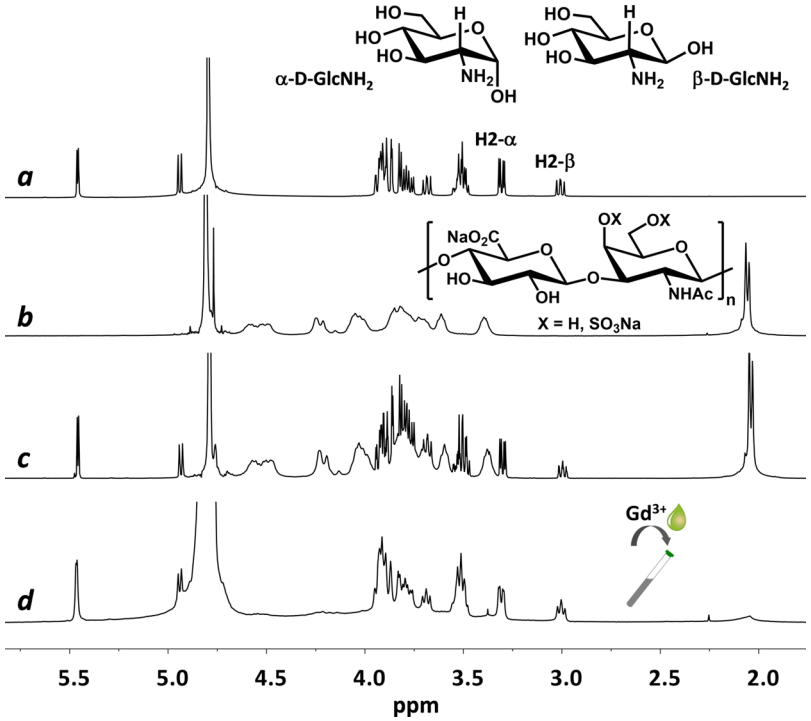

Figure 1. ^{1}H NMR spectra (D_2O, 500 MHz, 300 K) of $GlcNH_2$·HCl (10 mM) (a), ChS (10 mM disaccharide) (b), and a mixture of $GlcNH_2$·HCl (10 mM) and ChS (10 mM disaccharide) before (c) and after (d) the addition of Gd^{3+} (200 μM).

molecule/polymer (R_{2p} < 1000 s^{-1} mM^{-1}) by addition of μM concentrations of Gd^{3+} salts,[11] we envisioned an application of the PSR filter for the fast NMR screening of binding that avoids the necessity of previous synthetic manipulations. It was hypothesized that Gd^{3+} could facilitate the identification of species interacting with macromolecular receptors of high R_{2p} by selectively enhancing their R_2 values (via a receptor-mediated paramagnetic effect) while leaving unaffected the signals of noncompetitive binders.[14] As a result, strong broadening effects or complete suppressions should be expected, even for ligands that are too weak to generate changes in the chemical shifts or relaxation times in the absence of Gd^{3+}.

RESULTS AND DISCUSSION

To evaluate the viability of the PSR filter as an NMR screening technology, a sample composed of the oppositely charged polysaccharide chondroitin sulfate (ChS, R_{2p} 4616 s^{-1} mM^{-1}) and glucosamine ($GlcNH_2$·HCl, R_{2p} 59 s^{-1} mM^{-1}), a composition studied for the treatment of osteoarthritis,[15] was first considered as a model system. Figure 1a,b shows the ^{1}H NMR spectra of $GlcNH_2$·HCl (10 mM) and ChS (10 mM disaccharide). Interestingly, the spectrum of an equimolecular mixture of both components (10 mM each, Figure 1c) shows no change in the chemical shifts or line broadening in the signals of $GlcNH_2$ that could reveal the existence of an interaction with the polysaccharide. Comparison of the ^{1}H T_2 of $GlcNH_2$ (H2 in α- and β-isomers, H2-α and H2-β) before (R = 0; R is the ChS/$GlcNH_2$ molar ratio) and after (R = 1) mixing with ChS revealed virtually identical values in complete agreement with this statement (Figure 2a,b). Nevertheless, a radically different picture emerged after the addition of Gd^{3+} (200 μM) to these solutions. Adding Gd^{3+} to $GlcNH_2$·HCl resulted in an unaffected ^{1}H NMR spectrum of the monosaccharide without loss of resolution, as expected according to its small R_{2p} value (Figure 2c, spectrum with R = 0). Conversely, the addition of Gd^{3+} to the mixture produced a nearly complete suppression of the signals of ChS (compatible with its large R_{2p} value), accompanied by a significant broadening of the $GlcNH_2$ signals, an effect indicative of an electrostatic interaction between the components (Figure 1d). Indeed, analysis of the ^{1}H T_2 of $GlcNH_2$ in Figure 2a,b reveals drastic reductions in T_2 (more than 10-fold) when Gd^{3+} is added to the ChS/$GlcNH_2$ mixture, compared to only minor effects when it was added to the monosaccharide solution. A study on the variation of ^{1}H T_2 and signal resolution of $GlcNH_2$ in mixtures with increasing concentrations of ChS (Gd^{3+} fixed at 200 μM) confirms this effect at values of R as low as 0.1 (Figure 2). This example illustrates the potential and simplicity of the PSR filter in revealing binding interactions that are too weak to generate changes in the chemical shifts or relaxation times.

The feasibility of the PSR filter was then evaluated with intermolecular systems of interest in the pharmaceutical/biomedical fields and supramolecular chemistry. In the following sections, we describe its application for ligand screening in interpolymer complexes (IPCs) and a macromolecular cyclodextrin (CD) host. The technology is also revealed to be especially suited for the screening of DNA ligands owing to the high R_{2p} of the phosphated DNA backbone.

Interpolymer Complexes. The selective association of poly(carboxylic acids) and nonionic polymers [e.g., poly(ethylene glycol) (PEG), polyacrylamide, poly(*N*-isopropylacrylamide), or poly(vinyl alcohol)] via hydrogen bonds results in the formation of novel polymeric materials, known as interpolymer complexes (IPCs) with promising applications in drug delivery.[16] It has been reported that PEG (R_{2p} 22 s^{-1} mM^{-1}) forms pH-sensitive aggregates when associated with poly(acrylic acid)[17] (PAA, R_{2p} 3000−17 000 s^{-1} mM^{-1}

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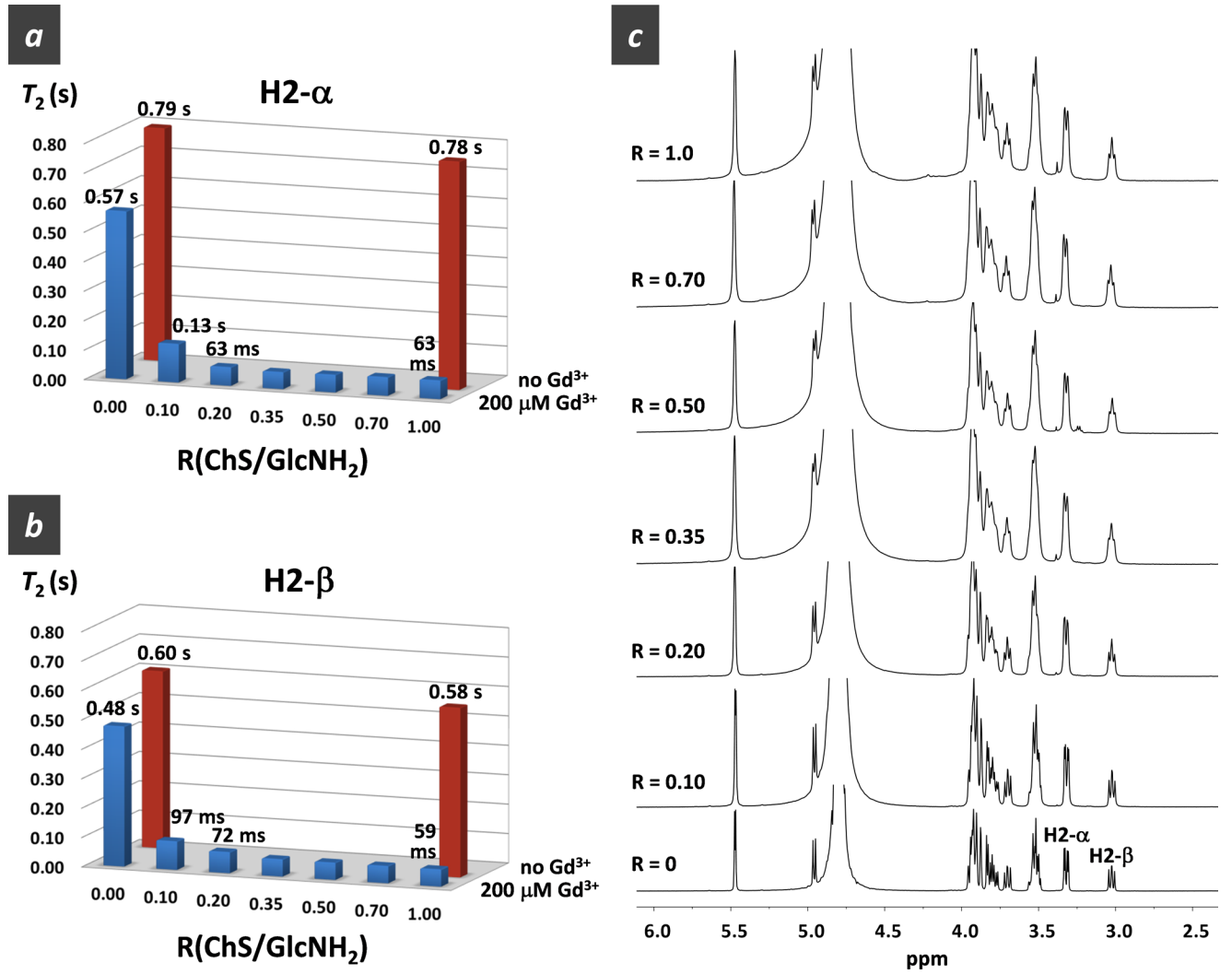

Figure 2. ^{1}H T_2 (D_2O, 500 MHz, 300 K) of $GlcNH_2$ (10 mM) [(a) H2-α, (b) H2-β] in mixtures with increasing proportions of ChS in the absence (red) and presence (blue) of Gd^{3+} (200 μM). (c) ^{1}H NMR spectra of ChS/$GlcNH_2$ mixtures in the presence of Gd^{3+} (200 μM); *R* represents the ChS/$GlcNH_2$ molar ratio.

depending on M_w). However, disclosure of this interaction is not evident by analysis of the ^{1}H NMR spectrum of the mixture (no signal broadening or variation of chemical shifts). Considering the high R_{2p} of poly(carboxylic acids) (R_{2p} > 1000 s^{-1} mM^{-1}), we envisioned the application of the PSR filter as an efficient strategy for the accelerated detection of IPCs using a standard ^{1}H NMR experiment.

Figure 3a shows the ^{1}H NMR spectrum of a ternary mixture composed of PAA_{450000} (0.3 mg/mL) and PEG_{5000} (0.3 mg/mL) forming an IPC, accompanied by $dextran_{66000}$ (0.75 mg/mL, R_{2p} 31 s^{-1} mM^{-1}) that does not participate in the association. The NMR spectrum shows the signals expected for the three individual components (broad signals for PAA at 1.5−2.6 ppm, a sharp singlet for PEG at 3.75 ppm, and various well resolved peaks around 3.5−4.0 ppm for dextran) but no evidence for the existence of an IPC. As predicted, the addition of Gd^{3+} (40 μM) to the mixture resulted in a nearly complete suppression of the components that participate in the IPC (PAA and PEG via direct and mediated paramagnetic effects, respectively) while leaving the resonances due to dextran unaffected (Figure 3b). A more efficient suppression of the IPC could even be obtained by the simultaneous implementation of a very short T_2-filter (e.g., Carr−Purcell−Meiboom−Gill (CPMG)), complementing the selective paramagnetic R_2 enhancement. As can be seen in Figure 3c, the broad residual signal from PEG observed in Figure 3b could be completely suppressed by application of a CPMG filter (10 ms), which does not affect the resonances of dextran, the component not participating in the IPC. We believe that the easy identification of the IPC by the PSR filter will facilitate the characterization of IPC-based hydrogels, layer-by-layer assemblies, and nanoparticles of interest in drug delivery and materials science.

Host−Guest Complexes. Cyclodextrins (CDs) are a family of cyclic oligosaccharides composed of a variable number of 1,4 linked α-D-glucopyranose units. Because CDs take the shape of a truncated cone with the central cavity having a relatively lipophilic character, they have found application in the food, cosmetic and pharmaceutical industries due to their ability to form inclusion complexes with a wide variety of hydrophobic guest molecules.[18] NMR is a privileged technique to detect and study complexes of CDs. The fastest approach relies on the observation of differences between the ^{1}H chemical shifts of the CD, guest, and the complex.[19] Unfortunately, signal overlapping and small variations in the

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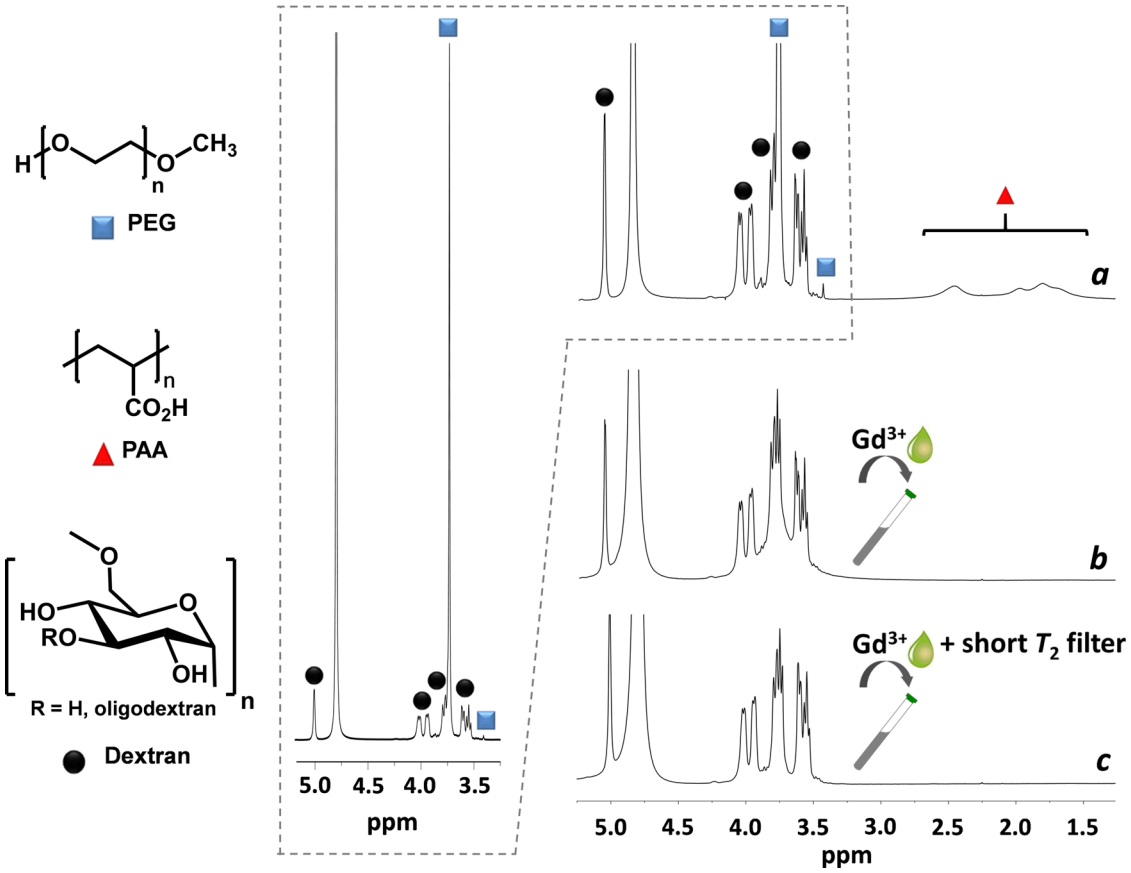

Figure 3. ^{1}H NMR spectra (D_2O, 500 MHz, 300 K) of a mixture of PAA_{450000} (0.3 mg/mL), PEG_{5000} (0.3 mg/mL), and $dextran_{66000}$ (0.75 mg/mL) before (a) and after (b) the addition of Gd^{3+} (40 μM), and after the addition of Gd^{3+} (40 μM) + T_2-filter (CPMG, 10 ms) (c).

chemical shifts often obscure an unambiguous identification of binding by ^{1}H NMR. Because of their limited solubility in water (especially β-CD, the most widely used member of the family), more soluble derivatives, including carboxylated and sulfated CDs, have been developed and are in common use.[20] Considering the large R_{2p} value of these macromolecular receptors [R_{2p} 1534 s^{-1} mM^{-1} for sulfated β-CD (sβ-CD)] compared to that of low molecular weight guest molecules, the PSR filter was envisaged as a convenient technology for the fast and easy detection of inclusion complexes, overcoming the aforementioned limitations of conventional ^{1}H NMR experiments.

As an illustrative example, the inclusion complex of sβ-CD and 1-adamantanol (AdOH, weak affinity ligand with K_D of ca. 650 μM)[21] was investigated in the presence of a noncompetitive binder, methyl-α-D-glucopyranoside (Glc-OMe). The experimental conditions for the preparation of the inclusion complex are described in the Supporting Information (SI). The small variations observed in the ^{1}H NMR spectrum of AdOH after binding hampered the identification of the inclusion complex, which was nevertheless clearly verified by a ^{1}H−^{1}H ROESY experiment (Figures S1 and S2 in the SI). Still, much easier and more direct proof of complexation came from application of the PSR filter. Figure 4a shows the ^{1}H NMR spectrum of an equimolecular mixture of sβ-CD, AdOH, and Glc-OMe, where characteristic signals due to the three components are clearly identified. As can be seen in Figure 4b, the simple addition of Gd^{3+} (400 μM) to the mixture allowed the easy identification of the sβ-CD/AdOH complex via selective broadening of their resonances while leaving completely unaffected those of Glc-OMe (linewidth and chemical shift). Note that in the absence of sβ-CD, the ^{1}H signals of AdOH are not affected by the addition of 400 μM Gd^{3+} (Figure S3). As in the example above, an even clearer picture of the selective complex formation was provided by the simultaneous implementation of a short CPMG filter (80 ms), which afforded a ^{1}H NMR spectrum of the nonbinding Glc-OMe ligand undistinguishable from that of the pure compound (Figure 4c; unattainable spectrum with CPMG filters in the absence of Gd^{3+}). This combined PSR−CPMG strategy was also applicable for the accelerated analysis of the 2D experiments ^{1}H−^{1}H COSY and ^{1}H−^{13}C HSQC of the mixture (Figure 4d−g), where identification of selective ligands is highly facilitated compared to that by using 1D experiments; a possibility envisioned to greatly facilitate the screening of large libraries of ligands.

Interestingly, when, for comparison purposes, NOE-based experiments were undertaken, they were unsuccessful in the identification of the sβ-CD/AdOH complex. Whereas Water-LOGSY[22] was inconclusive, saturation transfer difference (STD)[23] resulted only in internal transfer within sβ-CD. The outcome of these experiments unveils the advantage of PSR for the analysis of interactions with low molecular weight receptors, which, having short correlation times, lack an efficient distribution of magnetization through the spin system of dipolar coupled protons. Because PSR does not require selective saturation pulses, another advantage is its independence of spectral congestion, an important issue when dealing with large libraries of compounds.

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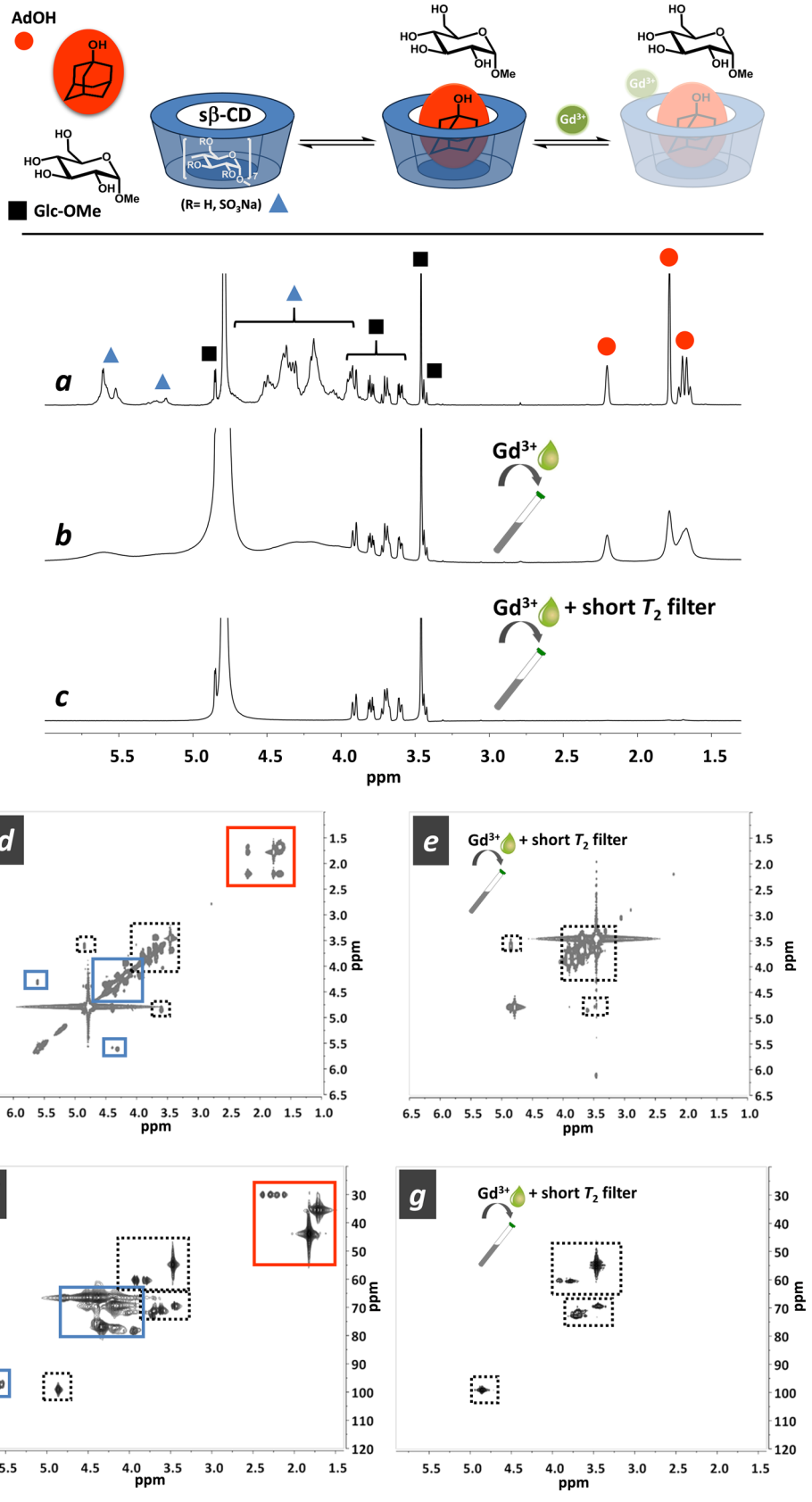

Figure 4. ^{1}H, COSY and HSQC spectra (D_2O, 500 MHz, 300 K) of an equimolecular mixture of sβ-CD, AdOH, and Glc-OMe (12 mg/mL) before (a, d, f) and after (b) the addition of Gd^{3+} (400 μM), and after the addition of Gd^{3+} (400 μM) + T_2-filter (CPMG, 80 ms) (c, e, and g).

DNA Ligands. Next, we proceed to evaluate this technology for the screening of DNA ligands. Because small molecules binding DNA interfere in essential processes like gene expression and replication, it is not surprising that they

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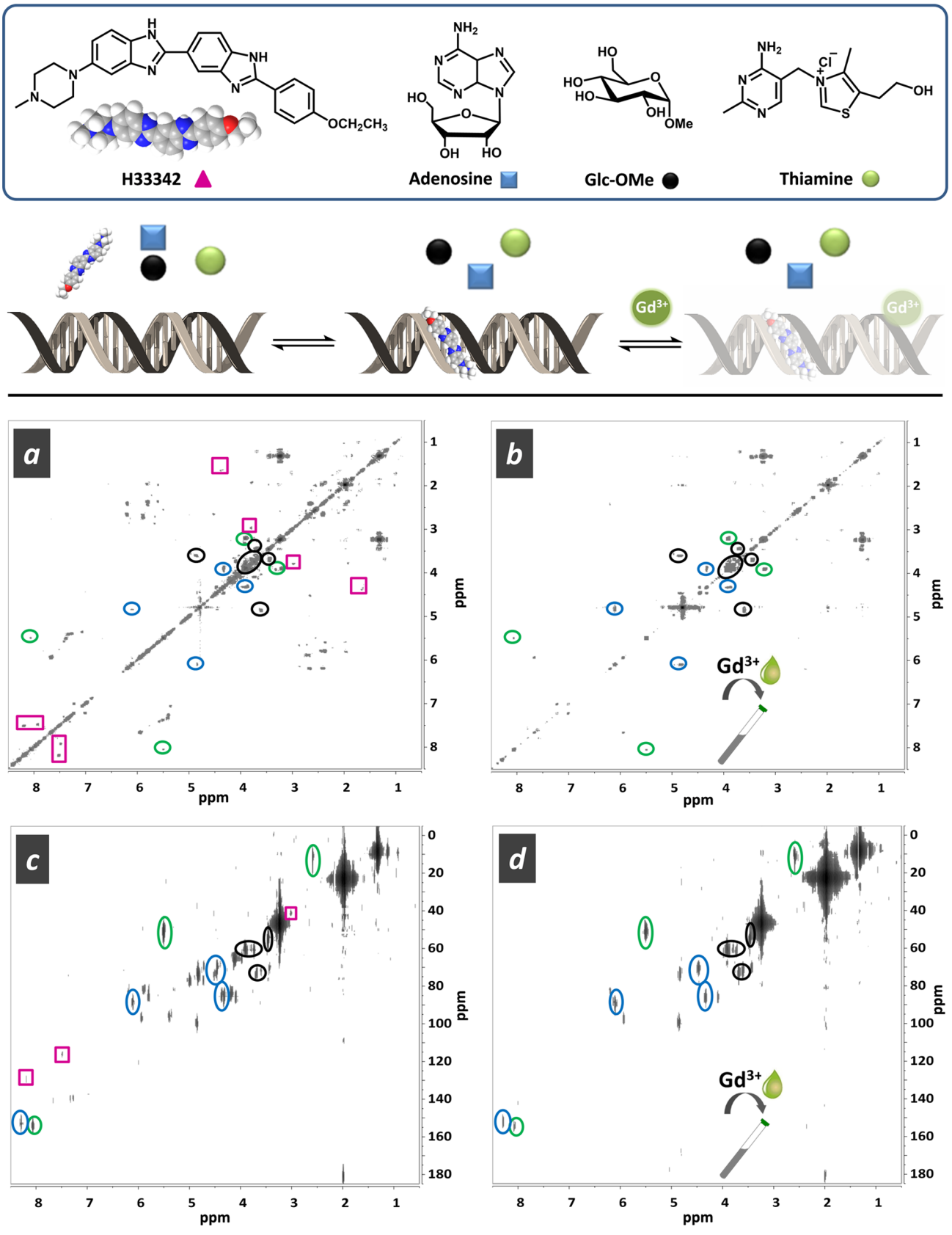

Figure 5. COSY and HSQC spectra (D_2O, 500 MHz, 300 K) of an equimolecular mixture of $d(CGCGAATTCGCG)_2$, thiamine, adenosine, Glc-OMe, and H33342 (0.6 mM each) before (a, c) and after (b, d) the addition of Gd^{3+} (30 μM).

represent an effective source of anticancer, antibiotic, and antiviral agents. DNA is nowadays the pharmacological target of many drugs that are able to recognize DNA surfaces, bind to specific regions, or intercalate at specific sequences.[24,25] In this

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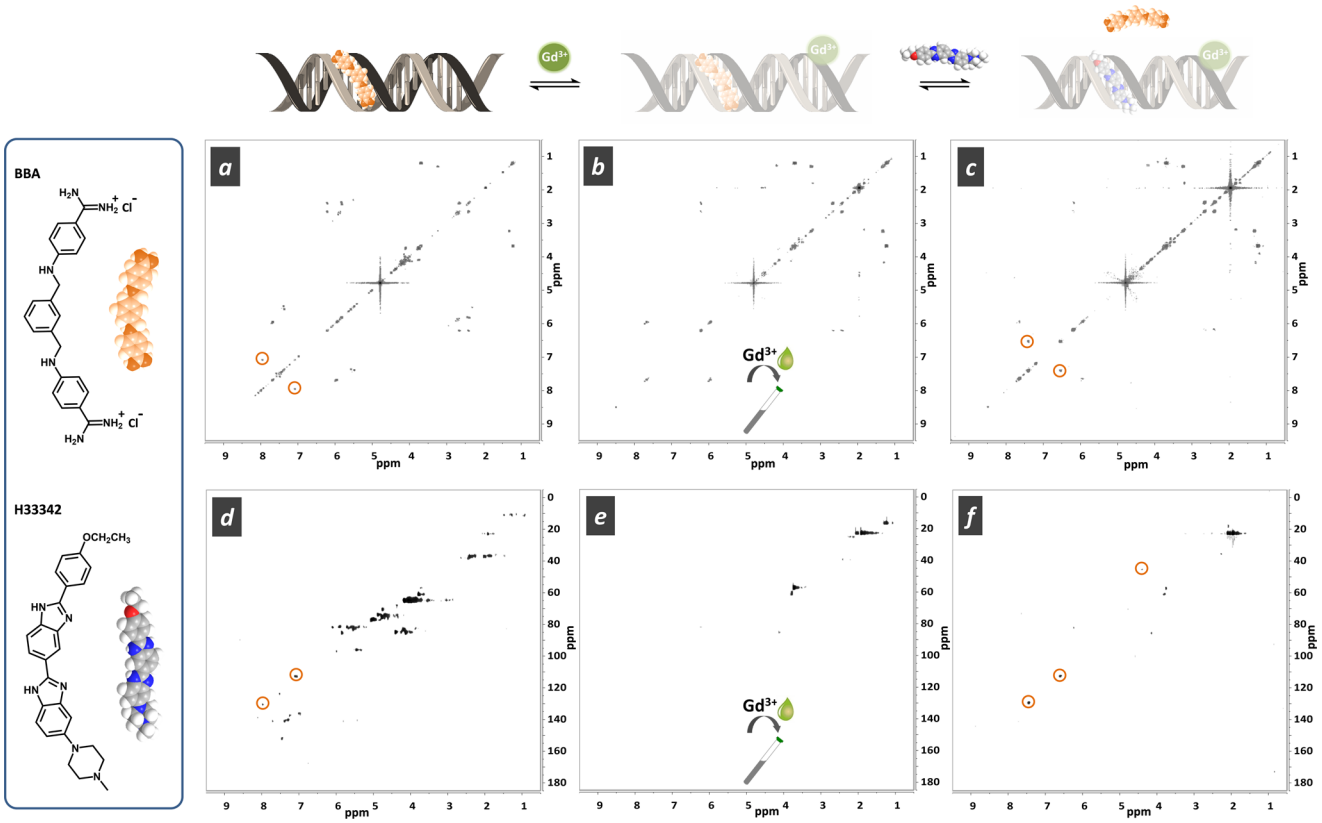

Figure 6. COSY and HSQC spectra (D_2O, 500 MHz, 300 K) of an equimolecular mixture of d(CGCGAATTCGCG)$_2$ and BBA (0.6 mM each) before (a, d) and after (b, e) the addition of Gd^{3+} (30 μM), and after a subsequent addition of H33342 (0.6 mM) (c, f).

context, NMR stands out as a robust tool for ligand screening.[25,26] Because the DNA backbone is composed of a phosphated deoxyribose pattern with excellent Gd^{3+}-complexing ability, the PSR filter was foreseen to facilitate the screening of DNA ligands over more established technologies.

To this end, we analyzed the selective binding of Hoechst 33342 (H33342, a strong minor groove ligand with K_D 14 nM)[27] to a duplex DNA dodecamer d(CGCGAATTCGCG)$_2$ in the presence of three nonbinding molecules, namely, thiamine, adenosine, and Glc-OMe.[28] Figure 5a,c shows the COSY and HSQC spectra (^{1}H NMR in Figure S4a) of an equimolecular mixture of dsDNA and the four ligands, which provide no clue about the selective binding of H33342 (verified via a T_2-filter experiment, Figure S5). Alternatively, the addition of minute amounts of Gd^{3+} (30 μM) to the sample afforded that information effortlessly. A series of spectra was obtained where the signals of H33342 had been selectively removed, leaving those of the nonbinding ligands untouched (Figures 5b,d and S4b). Both 2D experiments illustrate the vast selectivity and potential of the PSR filter for routine DNA screening of large libraries of compounds. For comparison purposes, when a STD was applied to the mixture, although the identification of H33342 was possible, the much higher sensitivity of PSR was revealed.

To discard false PSR positives from nonbinding molecules, which, having large Gd^{3+}-complexing abilities, could potentially lead to broadening effects or signal suppressions in the absence of binding, the dsDNA/H33342 system was evaluated under identical experimental conditions as above in the presence of glucuronic acid (R_{2p} 327 s^{-1} mM^{-1}), a non-DNA binder displaying one of the largest R_{2p} values described.[11] As expected, the addition of Gd^{3+} (30 μM) to an equimolecular mixture of the three components resulted in the selective and clean suppression of H33342 (COSY and HSQC) without affecting the signals of glucuronic acid (Figure S6). The fidelity of PSR as a screening technology was also challenged by a competitive experiment involving two ligands of dsDNA: H33342 (K_D 14 nM)[27] and a bisbenzamidine of lower affinity (BBA, K_D 724 nM).[29] Figure 6a,d shows the COSY and HSQC spectra of an equimolar mixture of dsDNA and BBA, where signals due to the ligand are clearly identified. The selective binding of BBA to dsDNA was easily confirmed via signal suppression after addition of Gd^{3+} (30 μM) (Figure 6b,e). A subsequent addition of an equimolecular amount of H33342 (higher affinity ligand) to the mixture resulted in a BBA to H33342 replacement in the minor groove, as evidenced by the reappearance of the BBA signals in both spectra (Figure 6c,f). Ultimately, uncomplexed BBA in solution is clearly visualized in the NMR spectra, whereas the signals of H33342 complexed in the minor groove are selectively suppressed via the receptor-mediated paramagnetic effect. This experiment confirms the potential of the PSR filter to monitor binding interactions in real time.

CONCLUSIONS

The paramagnetic spin relaxation (PSR) filter is described as a fast method for the NMR screening of intermolecular interactions. The addition of micromolar concentrations of Gd^{3+} to macromolecular receptors with large transverse relaxation enhancements (R_{2p}) is exploited for the suppression/broadening of the NMR signals of interacting ligands while leaving noncompetitive binders unaffected (1D and 2D

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experiments). The PSR filter affords rich screening information effortlessly, is operationally very simple, and so, it is amenable to ready implementation by nonspecialists. The high sensitivity of the method unveils interactions that are too weak to generate changes in chemical shifts or relaxation times. The feasibility of the PSR filter has been evaluated for ligand screening in interpolymer complexes and a macromolecular cyclodextrin host. In addition, it has been revealed to be especially suited for the screening of DNA ligands owing to the high R_{2p} of the phosphated DNA backbone. These examples demonstrate the reliability and broad applicability of the method for the fast NMR screening of intermolecular interactions. Applications to alternative macromolecular receptors and supramolecular structures are envisaged, including cages, calixarenes or peptide nanotubes, among others.

EXPERIMENTAL SECTION

Materials and Methods. All chemicals were purchased from commercial sources and used without further purification. $Gd_2(SO_4)_3{\cdot}8H_2O$ was purchased from Aldrich. D-Glucosamine hydrochloride, poly(acrylic acid) (M_v 450 000, by viscosity), poly(ethylene glycol) (M_n 4257, M_w 4867, by matrix-assisted laser desorption ionization time-of-flight), β-cyclodextrin sulfated sodium salt (sβ-CD), 1-adamantanol (AdOH), 2,5′-bi-1*H*-benzimidazole, 2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-trichloride (H33342), thiamine, and methyl-α-D-glucopyranoside (Glc-OMe) were purchased from Sigma. Adenosine was purchased from Merck. Dextran from *Leuconostoc mesenteroides* was purchased from Fluka (M_n 33 698, M_w 65 794, by gel permeation chromatography). The oligonucleotide Drew-Dickerson DNA dodecamer CGCGAATTCGCG was acquired from Thermo Scientific and biomers.net. Bisbenzamidine 4-([[3-([[(4-carbamimidoylphenyl)amino]methyl)phenyl) methyl]amino)benzene-1-carboximidamide (BBA) was kindly donated by Prof. M. Eugenio Vázquez (CIQUS, Universidade de Santiago de Compostela).[29] Condrosan (Bioibérica Farma) was used as the source of chondroitin sulfate. Each capsule of Condrosan contains approximately 82% of a mixture of chondroitin 4- and chondroitin 6-sulfate (M_w 14 000−18 000) and 18% magnesium stearate. The degree of sulfation of chondroitin sulfate was determined as 67% by elemental analysis (N: 2.91%; S: 4.49%) using a LECO Elemental Analyze Model CHNS-932.

NMR Spectroscopy. Reported R_{2p} values were determined at 8 mg/mL in D_2O (500 MHz) in the presence of either 13 μM or 1 mM Gd^{3+}.[11] NMR experiments were recorded on a Bruker Avance DRX-500 spectrometer of 11.7 T (^{1}H frequency 500 MHz), equipped with an inverse detection ^{1}H/X broadband BBI probe with z gradients and operating under Topspin 1.3 software. Chemical shift (δ) values are reported in ppm relative to the residual water peak (HOD; δ 4.79) used as an internal standard. ^{1}H−^{1}H COSY experiments were acquired in magnitude mode using the standard Bruker sequence "cosygp". ^{1}H−^{13}C HSQC experiments were recorded using the standard Bruker sequence "inviedgptp". The ^{1}H−^{1}H ROESY spectrum was obtained using a spin-lock time of 600 ms with the standard Bruker sequence "croesyprtp2".

^{1}H T_2 values were determined using the Carr−Purcell−Meiboom−Gill (CPMG) pulse sequence [$90°x - (\tau - 180°y - \tau)_n$, where 2τ is a fixed echo time (τ = 0.7 ms), n is the number of echoes, and $2\tau n$ is the total echo duration] using 16 values of t, where $t = 2\tau n$, with a minimum value of 1.4 ms (n = 1) and the maximum is about 6−7 times the highest T_2. Values of T_2 are averaged among 2−3 experiments. The interscan relaxation delay was larger than 5 times the highest ^{1}H T_1 in the sample. The absolute signal integral intensity (I) at each value of $2\tau n$ was fitted to the applicable monoexponential eq 1 to determine the relaxation time T_2.

$$I(t)/I_0 = \exp(-t/T_2) \quad (1)$$

1D and 2D T_2-edited experiments were performed by replacing the first 90° pulse by the CPMG pulse sequence as previously described,[30] using the same conditions as those described above (t = T_2-filter).

Mestre Nova 10.0.2 software (Mestrelab Research) was used for spectral processing. When comparing spectra, the same number of scans and apodization values were used. Residual HOD was attenuated in COSY experiments by processing. OriginPro 9.0 Software (Originlab Corporation) was used to perform the exponential fittings to obtain the relaxation times T_2.

Inclusion of 1-Adamantanol in sβ-CD in the Presence of Methyl-α-D-Glucopyranoside. In a test tube, sβ-CD (80 mg, 24.1 mmol), 1-adamantanol (3.36 mg, 24.0 mmol), and methyl-α-D-glucopyranoside (4.66 mg, 24.0 mmol) were mixed in Milli-Q H_2O (2 mL). The solution was kept under stirring for 6 h at room temperature and then it was freeze dried. Afterwards, 20 mg of the lyophilized solid was dissolved in 1 mL of D_2O, and 300 μL of this solution was transferred to an NMR tube. The final volume was made up to 500 μL with D_2O (final concentration of lyophilized mixture 12 mg/mL).

DNA Experiments. The oligonucleotide Drew-Dickerson DNA dodecamer CGCGAATTCGCG (7.6 mg) was dissolved in D_2O (760 μL) and heated at 95 °C for 10 min. This solution was allowed to slowly reach room temperature and it was used as the stock solution. For NMR experiments, 217 μL of the stock solution was transferred to NMR tubes, followed by a slow addition of the ligands dissolved in D_2O. Finally, D_2O was added to reach a 0.6 M solution of dsDNA and ligands.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b02074.

NMR experiments as Figures S1−S6 (PDF)

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Notes

The authors declare no competing financial interest.

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